

Identification of Vertebrate MMP-2 and MMP-9 like Molecules in the Aqueous Extract of Nasturtium (*Tropaeolum Majus*) Flowers, Bambusa Balcooa Leaves and Nayantara (*Catharanthus Roseus*) Flowers

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ABSTRACT

MMP-2 (activated) and MMP-9 like molecules which has similarity with vertebrate MMP-2 and 9 were identified in aqueous (PBS) extract of Nasturtium flowers, matured *Bambusa balcooa* leaves and Nayantara flowers. **Aims-** To search (MMP-2/MMP-9) Gelatinases like molecules from plant source. **Methods-** Zymography, Immunoblot, ELISA. **Conclusion-** The presence of activated MMP-2 and MMP-9 like molecules in plant source having similarity with vertebrate MMP-2 and MMP-9 may have academic and business potential.

Key words: Activated MMP-2; MMP-9; Activated; Flowers; Leaves; Plant

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INTRODUCTION

Matrix metalloproteinases (MMPs) are zinc dependant endopeptidases^[1-5]. It is present in diverse organisms from bacteria to multicellular vertebrates and invertebrates^[6, 7]. They are reported to degrade the ECM proteins^[8-12]. It is synthesised by connective tissues and has important functions in tissue degradation^[13]. Gelatinases is one of the groups of MMPs. The important members of this gelatinase family are Gelatinase-A (72 Kd) and Gelatinase-B (92 Kd), also called MMP-2 and MMP-9^[14-17]. MMPs (specially 2, 9) have special roles in cancer biology, metastasis and angiogenesis^[18-21]. MMPs role in the cell migration, cytoarchitecture, remodeling of connective tissues and wound healing and cancer biology have been reported^[22-38]. MMP-2 in several cancers has been reported to be associated with poor prognosis^[25-30]. Increased MMP-9 activity has been observed in several cancer cases compare to noninvasive counterpart^[39]. As per literatures MMP-2 and MMP-9 have potential to be cancer markers. In a very recent report^[20] the activated MMP-2 from breast cancer patients' saliva has been reported to be a potential marker for breast cancer using non-invasive method^[20]. There are 25-28 different types of MMPs^[25-30] reported so far. Studies on MMP biology was done mostly in vertebrates. In plants^[40-47] studies on MMP are very scanty and numbered. This study was to observe whether different parts of plants have MMP/gelatinase type of enzyme proteins.

In the present communication, we report that activated MMP-2 and MMP-9 like molecules which have appreciable similarity with vertebrate MMP-2 and MMP-9 are present in the plant source. This may have interesting academic and business potential.

MATERIALS AND METHODS

Nasturtium flowers, leaves from matured *Bambusa balcooa* and Nayantara flowers were collected and extracted for 2 hrs at 4°C in phosphate buffered saline (PBSX1, pH 7.4), centrifuged at 10,000x rpm for 30 mins at 4°C. Clear supernatants were saved. Proteins were estimated by Lowery's method. Acrylamide, Tris, SDS, Glycine, Gelatin etc were purchased from Sigma, USA. MMP-2, MMP-9, TIMP-1 and 2 monoclonal antibodies were purchased from Santa Cruz, USA.

Substrate Gel Electrophoresis (Zymography)

Equal amount (200 µg) of proteins from Nasturtium, *Bambusa balcooa* and Nayantara flowers PBS extracts were run in 8% SDS-PAGE impregnated with 0.1% Gelatin. The gel was run at 20 mA using Tris/Glycine/SDS buffer (pH 8.3). The gel was washed in 2.5% Triton-X for 1 hr and then incubated in buffer A (NaCl 0.2M, CaCl₂ 4.5 mM, Tris 50mM, pH 7.4) overnight at 37°C. The gel was stained with Coomassie Brilliant Blue to develop the zymogram^[20].

ELISA

To assay MMP-2, MMP-9, TIMP-1 & 2, 50 µg of proteins (from flowers and leaves PBS extract) were used to develop ELISA using respective monoclonal antibodies (MMP-2, MMP-9, TIMP-1 & 2) followed by 2nd antibody coupled to horseradish peroxidase (HRP). TMB was used as substrate. O.D was taken at 450 nm^[20].

Immunoblot Development

200 µg of proteins were run on 8% SDS-PAGE. Proteins were transferred onto nitrocellulose membrane and immunoblots were developed using monoclonal antibodies against MMP-2 and MMP-9 (Santa Cruz, USA) followed by alkaline phosphatase coupled 2nd antibody. The color was developed using NBT/BCIP^[20].

RESULTS

Figure 1 shows the zymogram of activated MMP-2 like molecule (72 KD) in the PBS extract of Nasturtium (*Tropaeolum majus*) flowers (1) and matured *Bambusa Balcooa* leaves (2) respectively. Figure 1-(3) shows the MMP-9 expression at 92 KD regions from the PBS extract of Nayantara flowers. When developed Immunoblots (Figure-2) with the monoclonal antibody of vertebrate MMP-2 and MMP-9 the Immunoblot of Nasturtium (1) and Bambusabalcooa (2) and Nayantara extract (3) (with vertebrate MMP-9 monoclonal antibody) very positive results indicate the similarities of MMP-2 and MMP-9 like molecules extracted from flowers and leaves showing band at 72 KD (MMP-2) and 92 KD (MMP-9) regions. The ELISA (Figure-3- 1, 2 & 3) were also positive reacted with vertebrate MMP-2 (1&2) and MMP-9 (3) antibodies. Figure-4 shows the ELISA result of Nasturtium and *Bambusa balcooa* PBS extracts using vertebrate TIMP-2 (1 & 2) and Nayantara flower extract with vertebrate TIMP-1 (3) monoclonal antibody (Santa Cruz, USA).

DISCUSSION

MMPs especially MMP-2 and MMP-9 have important roles in development, cytoarchitecture, wound healing etc. Most of the

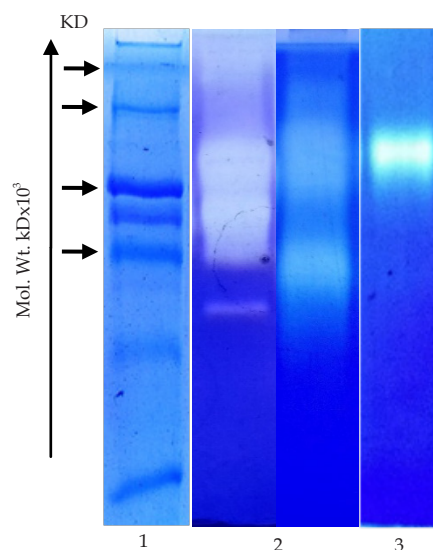


Figure 1 Zymography of PBS extract of (1) Nasturtium (2) Bambusa balcooa (3) Nayantara flowers extract 200 µg of proteins extract was run on 8% Acrylamide gel impregnated with 0.1% gelatin in Tris-Glycine-SDS buffer, pH 8.3 at 20 mA. The gel was washed in 2.5% Triton-X-100 for 1 hr and kept at 37°C in reaction buffer overnight in a 37°C incubator. Next morning gel was stained with 0.3% Coomassie Blue.

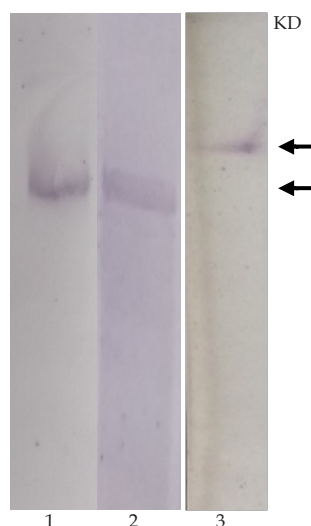


Figure 2 Immunoblot of PBS extract of (1) Nasturtium (2) Bambusa balcooa (3) Nayantara flowers extract. 200 µg of proteins were run in acrylamide gels. Proteins were transferred onto a nitrocellulose membrane. The membrane was reacted with vertebrate MMP-2 and MMP-9 (Human/ mouse) monoclonal antibodies followed by alkaline phosphatase coupled 2nd antibody. The colour was developed with NBT/BCIP substrate.

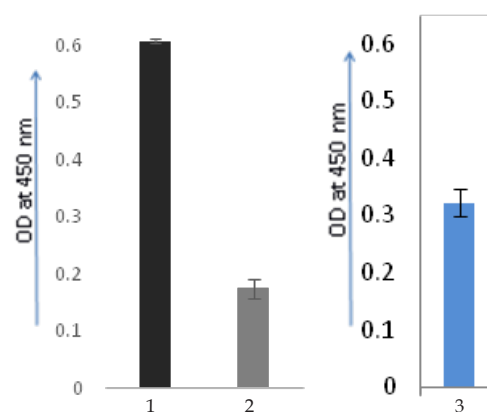


Figure 3 ELISA of PBS extract of (1) Nasturtium (2) Bambusa balcooa (3) Nayantara flower extract. 50µg proteins were used to develop ELISA using respective monoclonal antibodies (MMP-2 or MMP-9). Followed by HRP coupled 2nd antibody was used. Colour was developed using TMB as substrate and read at 450 nm.

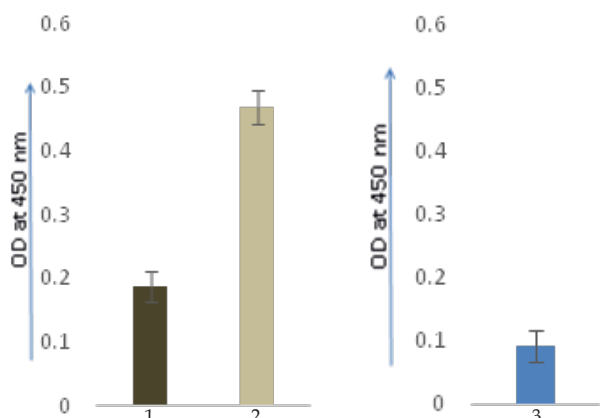


Figure 4 TIMP-2 ELISA of PBS extract of (1) Nasturtium (2) Bambusa balcooa. TIMP-1 ELISA of PBS extract of Nayantara flower extract using TIMP-2 and TIMP-1 monoclonal antibodies respectively followed by 2nd antibody coupled to HRP. TMB was used as substrate. Colour was taken at 450 nm.

known MMPs have been studied in vertebrates. The role of MMP-2 and 9 like gelatinases in cancer biology is well referred^[22-36]. The studies on MMP like molecules are scanty in the plant world^[40-47]. To understand whether MMPs/gelatinase like enzyme protein express in plant world we extracted several flowers and leaves of different plants in Phosphate buffered saline X1, pH 7.4. In three of them (Nasturtium, matured *Bambusa balcooa* and Nayantara) we observed that the MMP-2 (in Nasturtium flowers and *Bamboosa balcooa* leaves) and MMP-9 (in Nayantara flowers) expression is very strong in zymogram. To identify the species we cross reacted the plant extracts (Nasturtium, *Bambusa balcooa* and Nayantara flowers) with the monoclonal antibodies against human/ mouse MMP-2 and MMP-9 (Santa Cruz, USA) developing ELISA and Immunoblots. The results show very positive reactions indicating that the PBS extracts of flowers & leaves (Nasturtium & matured *Bambusa balcooa*) and Nayantara (flowers) are cross reacting with vertebrate (Human/Mouse) MMP-2 (Nusturtium and *Bambusa balcooa*) and MMP-9 (Nayantara). The observation may indicate that MMP-2 and MMP-9 like molecules are conserved in nature. One of the important observations is that this plant derived MMP-2 is in activated form showing 3-4 activated bands (72, 64, 55 KD) in Nasturtium flower extract and 2 bands in matured *Bambusa balcooa* (72, 64 KD) leaves extracts. Observing the presence of MMP-2 (activated) like molecule in plant extracts we were interested to see whether the same extracts have presence of TIMP-2 like molecule which is natural inhibitor of MMP-2. From our ELISA results we observed that TIMP-2 like molecule is present in Nasturtium and *Bambusa balcooa* extracts. These observations strongly indicate the presence and role of this vertebrate MMP-2 like molecule in flowers (Nasturtium) and leaves (*Bambusa balcooa*) aqueous extracts. Similarly Nayantara flower extract shows very strong band at 92 KD regions (Figure-1-3) in the zymogram and gives ELISA and blot positive results with vertebrate human/ mouse MMP-9 monoclonal antibody. The Nasturtium and matured *Bamboosa balcooa* extracts were also positive in immunoblots with MMP-2 antibody and Nayantara with MMP-9 antibody. It may also strongly indicate that this vertebrate MMP-2 (activated) and MMP-9 like molecules may have some important roles in the plant morphology, cytoarchitecture and developemnt. It may also have strong academic and industrial potential. More characterization of the molecules are in progress.

CONCLUSION

Aqueous (PBS) extracts of Nasturtium flowers, *Bambusa balcooa* leaves and Nayantara flowers show the presence of activated MMP-2 and MMP-9 like molecules having similarity with vertebrate (Human/ mouse) activated MMP-2 and MMP-9. The observations may have academic and industrial potential.

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